THESIS

PARASITES OF TWO CLOSELY RELATED POECILIID SPECIES ACROSS A SALINITY GRADIENT ON THE ISLAND OF TRINIDAD: IMPLICATIONS FOR GEOGRAPHIC RANGE LIMITS

Submitted by

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ABSTRACT

PARASITES OF TWO CLOSELY RELATED POECILIID SPECIES ACROSS A SALINITY GRADIENT ON THE ISLAND OF TRINIDAD: IMPLICATIONS FOR GEOGRAPHIC RANGE LIMITS

Parasite communities can vary greatly both within and amongst host populations. Many factors may be responsible for this variation in parasite diversity, and parasite-host relationships are of great ecological importance as parasites can alter host behavior, impact population demography, and drive co-evolutionary dynamics. One long-standing ecological question is how these parasite-host interactions shape mutual geographic distributions, which are also impacted by various abiotic and biotic factors. However, few studies have investigated how parasite communities change across environmental gradients and different host species, or how parasite abundance changes within and outside the host geographic range.

The island of Trinidad provides a model system that can be used to address these questions. On this island, the tropical fish *Poecilia reticulata* inhabits mountainous and lowland freshwater streams but avoids brackish waters. A close relative, *Poecilia picta* inhabits both lowland freshwater and brackish water streams. To date, no study has investigated how internal parasite communities vary across this salinity gradient or between these two closely related host species with overlapping geographic ranges. In lab studies, *P. reticulata* has been shown to be physiologically tolerant of brackish water, suggesting some other environmental factor may limit their dispersal and range expansion into brackish waters. Here we investigated how internal parasite diversity changes between 1) natural *P. reticulata* and *P. picta* populations in freshwater,

2) natural populations of *P. picta* in fresh and brackish water sites, and3) *P. reticulata*, experimentally exposed to fresh and brackish water conditions.

We found the prevalence of digenean trematode metacercariae to be 100% across three different river systems for both host species, however, mean metacercarial abundance differed significantly by river. Based on morphological differences in the metacercariae, we identified three distinct morphospecies. All three morphospecies were found in freshwater *P. reticulata* and *P. picta*. However, mean abundance of parasites varied across the two host species with *P. reticulata* harboring more parasites, on average, compared to *P. picta*. All three morphospecies were also found in *P. picta* in brackish water, but the total mean metacercarial abundance of *P. picta* was found to be increased in brackish compared to freshwater sites. Collectively, these results suggest that the three morphospecies utilize both hosts and they are limited in their geographic distribution by salinity. Still, the internal abundance of parasites varies between the two hosts depending on the salinity.

We tested whether these same parasites may be limiting the distribution of *P. reticulata* to freshwater, by experimentally exposing *P. reticulata* populations to field-collected brackish water. We assumed field-collected brackish water contains live cercaria and conducted exposures for a period of seven days. Compared to controls exposed to field-collected freshwater, there was a significant increase in the internal metacercarial abundance, along with an increase in mortality amongst the brackish water exposed group. These results suggest that movement of *P. reticulata* into novel brackish environments may be inhibited by increased parasitism; however, further investigations are warranted to better understand the mechanisms that determine the geographic distributions of parasites and their hosts.

ACKNOWLEDGEMENTS

I would like to acknowledge everyone who has played a role in my academic accomplishments: My friends and family for their unwavering love and support and my committee members who provided support and guidance through the research process.

DEDICATION

This thesis is dedicated to my mother, Rita, my father, Jeff, my partner in crime, Eric, and my special friend Twinkie.

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INTRODUCTION

Understanding the factors that shape geographic distributions of species is a central question in evolutionary ecology (Gaston 2009; Sexton et al. 2009; Bozick and Real 2015; Heads, 2015). Yet, progress towards answering why a species has a given geographic distribution remains a fundamental challenge, and the ecological, physiological, and evolutionary causes of range limits remain untested for most groups of organisms (Dynesius and Janssen; 2000; Paradis et al., 2002; Gaston 2009; Sexton et al. 2009). In theory, geographic range limits occur when environmental conditions reduce population size through a reduction in survival, reproduction, or emigration (reviewed in Gaston 2009). Typically, studies investigating the causes of species distribution focus on abiotic factors such as temperature, salinity, and rainfall (Van der Putten et al., 2010). Biotic interactions such as predation, food availability, and parasitism are known to play a role in a species distribution as well (e.g. Price and Kirkpatrick 2009; Wisz et al., 2012). However, while it is generally acknowledged that geographic range limits should be shaped by the combined influence of abiotic and biotic factors, few studies have explored how multiple factors interact to influence population dynamics at the range boundaries (Gaston 2009).

Parasites are some of the most abundant organisms on earth (Windsor, 1998), and their intimate relationship with host species pose unique challenges when examining the factors that shape their geographic distributions (Horwitz and Wilcox, 2005; Bozick and Real 2015). Parasites are simultaneously dependent on their hosts to complete their life cycles, while also potentially negatively impacting host health, survival (Ebert, 2005), and fecundity (Minchella and Scott, 1991). Because parasites will spend at least a portion of their lives on or within their

host(s), distribution and diversity of potential host species is closely tied to the distribution and diversity of parasite assemblages within a host population (Blasco-Costa and Poulin, 2013). Thus, while parasites present an interesting system to investigate how distributions change across environmental gradients and different host species, little is known about how parasite assemblages change within and across host species occupying a range of environments.

The host specificity and life cycle of parasites ultimately determine their geographic distributions, but spatial distributions of parasites cannot easily be predicted by simply examining the distribution of their hosts (Bozick and Real 2015). Parasites exhibit varying levels of specificity when it comes to suitable host species (Galaktionov and Dobrovol'skiĭ, 2003). While some species can infect a wide range of host species (i.e. a parasite generalist), others may only be suited for one host species at a certain life cycle stage (i.e. a parasite specialist) (Fried and Graczyk, 1997). For instance, among the digenean trematodes in the genus *Diplostomum*, some species are highly specialized to only infect the eye lens of their fish hosts, but are considered generalists with regard to their hosts, and infect a wide range of fish species. In contrast, other *Diplostomum* species that infect other tissues are known to parasitize only a few closely related fish host species and are thus considered host specialists (Locke et al., 2010). Despite such characterizations, the relationship between host and parasite geographic distributions is less than clear; for example, a parasite may infect a number of host species but be limited to a narrow geographic area, or infect a single host that occupies a broad geographic area (Bozick and Real 2015). Unlike many other organisms, the host environment may act to buffer the parasite against changes in the external environment, which may either limit or broaden the geographic distribution of a parasite (Cwiklinski et al. 2014). For example, internal parasites embedded within the host's tissue (e.g. liver, muscle) are buffered from the external environment

compared to external parasites (e.g. skin, scales). Parasites that encyst within the tissues of their hosts (e.g. the formation of metacercaria) may be even further buffered from the external environment and the hosts' immune system (Martinez and Merino, 2011). In aquatic species, the primary stage of the life cycle where the external environment has the greatest potential to act directly on the parasite is during the free-swimming stage (e.g. cercaria), when parasites move between host species. Yet, relatively little is known about how variation in the external environment and the internal host environment alter the distribution of different parasite species (Mostowy and Engelstadter, 2011).

Digenean trematodes, a group of parasites with complex multi-host life cycles that often utilize both aquatic and terrestrial environments to complete their life cycle (Galaktionov and Dobrovol'skiĭ, 2003), provide a model system to investigate the correlates of parasite geographic range limits. Digenean trematodes are a common parasite of freshwater and saltwater fish species, and have been shown to impact host species in several ways (Probst and Kube, 1999). For instance, several physiological changes can be attributed to digenean infection such as alteration of metabolic rate (Minguez et al., 2012), neuromodulation (Lafferty and Shaw, 2013) and reduction in reproductive tissues (Hurd, 1990). Additionally, environmental factors such as temperature (Thieltges and Rick, 2006) and salinity (Lei and Poulin, 2011) have been shown to influence digenean infection rates and development within aquatic environments.

Here we ask how digenean trematode assemblages change within and across host species and across a freshwater-saltwater gradient. We focused on two closely related host fish species, *Poecilia reticulata* and *P. picta* occupying lowland freshwater and brackish water rivers on the island of Trinidad. Populations of *P. reticulata* are restricted to freshwater sections of lowland rivers, whereas their sister species, *P. picta,* inhabits both fresh and brackish waters on the island

(Torres-Dowdall et al. 2013). This system allowed us to ask: 1) Do digenean trematodes exhibit host species preference in a freshwater environment, and 2) Do digenean trematode infection rates differ between *P. picta* in freshwater and brackish water environments. If *P. reticulata* and *P. picta* share the same set of parasites and have similar infection rates within freshwater, we would conclude the distribution of the parasites is not limited by their host species. Similarly, if we find no difference in the parasites of *P. picta* found in fresh and brackish water we would conclude that salinity does not pose a barrier to the distribution of the parasites.

MATERIALS AND METHODS

COLLECTION AND PARASITE ANALYSIS

We collected *P. picta* and *P. reticulata* from three river systems on the island of Trinidad: the Caroni, Caparo, and Cunupia rivers (Figure 1.1). To test if parasite assemblages differed between *P. picta* and *P. reticulata* where they coexist in freshwater, we sampled localities that were within a kilometer upstream of the fresh/brackish water interface in the Caroni, Caparo and Cunupia rivers. To test if parasite composition differed between *P. picta* populations inhabiting freshwater and brackish water sites, we compared the freshwater populations in the Caroni and Cunupia rivers to adjacent downstream locations: The Cunupia river sampled at 8ppt salinity, and the Caroni river sampled at 4ppt salinity (Figure 1.1).

At each site, we collected adult female Poeciliids from the banks of each river utilizing butterfly nets. Because *P. reticulata* and *P. picta* are small fish, we focused on adult females ranging from 2 to 3 cm, because they are larger than males and provided a potentially larger habitat for parasites to target. All fish were euthanized utilizing MS-222 just prior to analysis. Weight of the fish was obtained utilizing an Ohaus SP-602 Scout Pro Digital Balance, and standard length was obtained by placing the individual on a ruler and taking a digital picture with

an embedded scale. We used the software package ImageJ (Rueden et al. 2017) to calculate standard length. Individuals were analyzed microscopically for parasite species present and for total parasite abundance through fin clippings of pectoral and caudal fins, skin scrapings, whole gill squash preps, squash preps of abdominal viscera (GI tract, liver and gonads) using a compound microscope [American Optical fiftyOne] (Yanong 2003, Leung and Poulin 2011, Weber and Govett 2009). Due to the overwhelming abundance of visceral digenean trematode metacercariae present in both host species, we used this stage of the life-cycle as the focus of our survey.

In the process of quantifying metacercariae prevalence (presence or absence) and abundance (number of parasites per individual) (Bush et al. 1997), we noted three distinct morphological types (hereafter morphospecies). Metacercariae were easily categorized into three morphospecies based on size and characteristics noted by light microscopy (Figure 1.2). Morphospecies 1 was 2,418.58 microns on average and spherical with an "X" shaped excretory duct. Morphospecies 2 was 475.86 microns across with oral spines. Morphospecies 3 was ovoid (2,421.03 by 1,217.89 microns) and had a distinct anterior and posterior region.

STATISTICAL ANALYSIS

All analyses were performed in R 3.3.1. Parasite prevalence and mean abundance were quantified to assess how the parasite assemblage changed across host populations and across fresh and brackish water sites. We compared how the parasite prevalence changed across the two host species and between fresh and brackish water sites by comparing the percentage of fish parasitized by each of the morphospecies.

In order to test if parasite abundance differed between host species we used a generalized linear model (GLM) using the total abundance of visceral metacercariae as the response variable

(i.e. all three morphospecies combined), and host species, and population as predictor variables. We then ran the same model for each morphospecies separately to test if they differed between the two hosts.

To test if parasite abundance differed between fresh and brackish water we used a generalized linear model with total parasite abundance as a response variable and host location and population as predictor variables. As above, we then ran the same model for each morphospecies separately. In all cases, fish mass and length were not found to be significant predictors of parasite mean abundance and thus were not included in any model. We used a Poisson distribution in all our models because parasite abundance was not normally distributed and skewed with some individuals having very high abundances and the majority having few (see Figure 1.3), which is typical of parasite count data (Anderson and Gordon, 1982).

RESULTS

METACERCARIAL MORPHOSPECIES PREVALENCE

When the total metacercaria abundance of all three morphospecies combined was examined, we found *P. reticulata* and *P. picta* had one hundred percent prevalence rates across all three drainages examined (Figure 1.4). However, the percentages of each morphospecies varied between hosts. Morphospecies 2 was the most common as it was found in all rivers and in both *P. reticulata* and *P. picta* (Figure 1.4). Morphospecies 1 had relatively low prevalence rates among *P. reticulata* populations compared to that of *P. picta* in both freshwater populations, while morphospecies 3 had a higher prevalence rate among *P. reticulata* compared to that of *P. Pict* across both populations compared (Figure 1.4).

When we compared the mean prevalence of morphospecies within *P. picta* between the freshwater and brackish water types, again we found morphospecies 2 had one hundred percent

prevalence in both freshwater and brackish water (Figure 1.5). Morphospecies 1and 3 had relatively high prevalence with a trend to be higher in freshwater compared to brackish water (Figure 1.5).

METACERCARIAL MORPHOSPECIES ABUNDANCE

Where *P. picta* and *P. reticulata* were sampled together in freshwater the total metacercarial abundances differed significantly between the two host species ($z=2.37$, $df=1$, $p<0.017$) and between drainages ($z=4.52$, $df=2$, $p<.0001$), while there was no host x drainage interaction ($z=-0.58$, $df=2$, $p=0.56$). When we tested each morphospecies separately, we found morphospecies 2 was by far the most abundant parasite in freshwater for both host species, but was significantly more common in *P. reticulata* than *P. picta* ($z=2.85$, $df=1$, $p<0.001$; see Figure 1.6). Morphospecies 2 differed significantly between drainages ($z=4.83$, $df=2$, $p<0.0001$) however there was no host x population interaction ($z=-0.96$, $df=2$, $p=0.34$). In contrast, morphospecies 1 and 3 were not found to be different between host species (Figure 1.6), while the drainages differed for morphospecies 3 ($z=2.3$, $df=2$, $p=0.02$), but not morphospecies 1 ($z=-1.22$, $df=2$, $p=0.22$). No drainage x host relationship was detected for morphospecies 1 or 3.

When we tested the effect of salinity on relative overall metacercarial abundance between *P. picta* populations in fresh and brackish water we found a significantly higher parasite abundances in brackish water ($z=-8.4$, $df=1$, $p<0.0001$), and this difference varied between drainages ($z=-6.14$, $df=1$, $p<0.0001$). Additionally, we found a drainage x salinity interaction ($z=7.02$, $df=1$, $p<0.0001$). When we examined each morphospecies separately, we again found morphospecies 2 to be the most abundant parasite (Figure 1.7), with a significantly higher mean abundance in brackish water populations ($z=-8.4$, $df=1$, $p<0.0001$; Figure 1.7). However, we also found these differences varied amongst drainages ($z=-5.3$, $df=1$, $p<0.0001$) and detected a

population x salinity interaction ($z=6.73$, $df=1$, $p<0.0001$). There was no difference between freshwater and brackish water populations for morphospecies 1 or 3 (Figure 1.7), additionally we found these differences varied amongst drainages for morphospecies 1 ($z=-3.12$, $df=1$, $p<0.01$).

DISCUSSION

Due to their complex life cycles, the propagation and dispersal of digenean trematodes is highly linked to their hosts dispersal and ecology (Maze-Guilmo et al., 2016). Yet, parasites and their hosts may be constrained in their distributions by different environmental factors. In this study, we found that based on morphological characteristics there are three primary digenean trematodes able to parasitize both *P. reticulata* and *P. picta* in freshwater. These same parasites are also found in *P. picta* populations in fresh and brackish water environments. Thus, differences in host species and differences in salinity do not appear to limit the distribution of these parasites. However, the abundance of each morphospecies did vary depending on the host and salinity levels, suggesting hosts and salinity levels are not equally optimal environments. Morphospecies 2 was far more abundant in *P. reticulata* than *P. picta* where they coexist in freshwater (Figure 1.6), suggesting *P. reticulata* is a better host species in freshwater. However, in *P. picta*, Morphospecies 2 is significantly more abundant in brackish water compared to freshwater populations (Figure. 1.7). Such results suggest an interesting interaction between the host species (*P. reticulata* vs. *P. picta*) and the external salinity (fresh vs. brackish). Below, we discuss these and other patterns in more detail.

We found three morphologically distinct metacercaria within *P. reticulata* and *P. picta*. While there have been extensive studies of the monogenean parasite *Gyrodactylus* on Trinidadian Poeciliids (Harris and Lyles, 1992; Scott and Anderson, 1989; Dargent et al. 2013), to our knowledge this is the first description of Digenean trematodes in Poeciliids on the island

of Trinidad. We have yet to confirm the species of these trematodes, but based on the morphological features we suspect morphospecies 1 is in the genus *Centrocestus*, morphospecies 2 is in the Order Echinostome, and Morphospecies 3 is in the genus *Posthodiplostomum* (Galaktionov 2003, Skrjabin 1964). Future work is needed to confirm the identity of the three morphospecies, and characterizing the life cycle of these parasites also remains a major goal. For example, we do not know the species that serve as definitive and first intermediate hosts for the three morphospecies examined. How these hosts are distributed along the freshwater-brackish water continuum and the degree to which the different morphospecies utilize these hosts has obvious implications for shaping the distribution of the parasites. We also do not know if what we are describing as a single morphospecies is in fact made up of more than one cryptic species (e.g. Hebert et al. 2004), which may actually vary between host species or between fresh and brackish water. For the purposes of this study we assume each morphospecies is the same, but future DNA barcoding is being planned to test this assumption.

Digenean trematodes of fish can be host specialists or generalists (Galaktionov and Dobrovol'skiĭ, 2003). We found that where these two closely related host species coexist in freshwater, all three parasite morphospecies were detected in both hosts, however the prevalence of each morphospecies varied (Figure 1.4). For example, all individuals sampled had 100% prevalence rate of metacercarial morphospecies 2, whereas the prevalence of morphospecies 1 and 3 differed between the two closely related host species (Figure 1.4). Prevalence was not a good predictor of parasite abundance. For example, despite equal prevalence in the two host species, morphospecies 2 achieves significantly higher abundance in *P. reticulata* (Figure 1.6). This variation in morphospecies prevalence and abundance among the coexisting host species could indicate a difference in host preference by each parasite (Galaktionov and Dobrovol'skiĭ,

2003), the suitability of each host (Stokke et. al, 2018), or may indicate a difference in exposure rates of the host species to infective stages of digenean trematodes (Webster et. al, 2017). Morphospecies preference for their host may be due to differences in resistance or immune activity amongst hosts, or even differences in host behavior that may expose one host to infective cercariae more than another (Klemme and Karvonen, 2016). Despite the variation between the two hosts, these results indicate that these parasite morphospecies are not constrained by host type in freshwater where the two host species coexist.

Fish host species that inhabit the freshwater-brackish water interface typically do not harbor as many, or the variety of parasites species, compared to their freshwater or saltwater counterparts (Moller, 1978). The degree to which salinity poses a barrier to the movement or survival of free-living cercaria relative to their hosts should determine the geographic range limits of parasites (Moller 1978). We found that all three morphospecies were present in *P. picta* populations occupying fresh and brackish water (Figure 1.5, 1.7), suggesting both the parasites and the host species are tolerant of a wide range of salinities. We observed variation among morphospecies in that *P. picta* populations were less commonly infected in brackish water (i.e. they had lower prevalence) than their freshwater counterparts (Figure 1.5). However, when these brackish water individuals are infected they tend to harbor more parasites than their freshwater counterparts (Figure 1.7). One hypothesis for why individuals in brackish water have more parasites could be related to the hormonal control of osmoregulation. Cortisol plays a key role in osmoregulation of euryhaline fish (McCormick et al., 1989; Gonzalez, 2012), and has also been linked to decreased immune response to pathogens (Espelid et al., 1996) by influencing lymphocyte numbers and antibody production capacity (Barton, 1991). Thus, these results could indicate that exposure to brackish water may decrease a host's ability to ward off cercarial

infection. An alternative hypothesis is that the free-living stage of these parasites (cercariae) are simply more common in brackish environments leading to differences in exposure between the two environments.

CONCLUSIONS

The geographic distributions of parasites are shaped by a variety of limiting factors (Bozick and Real 2015). Here we asked if different host species occupying a gradient from freshwater to brackish differed in their parasite assemblages. We found three distinct Digenean trematodes morphospecies, and while there was variation in the prevalence and abundance of each morphospecies across the hosts and the different levels of salinity, there was no evidence that any of these factors limit the distribution of the parasites. These results serve as a first step in answering this question, and further study is needed to test whether salinity has any influence on the life cycle of the parasites, and the degree to which the parasites and their hosts may show population level differences between fresh and brackish water.

FIGURES

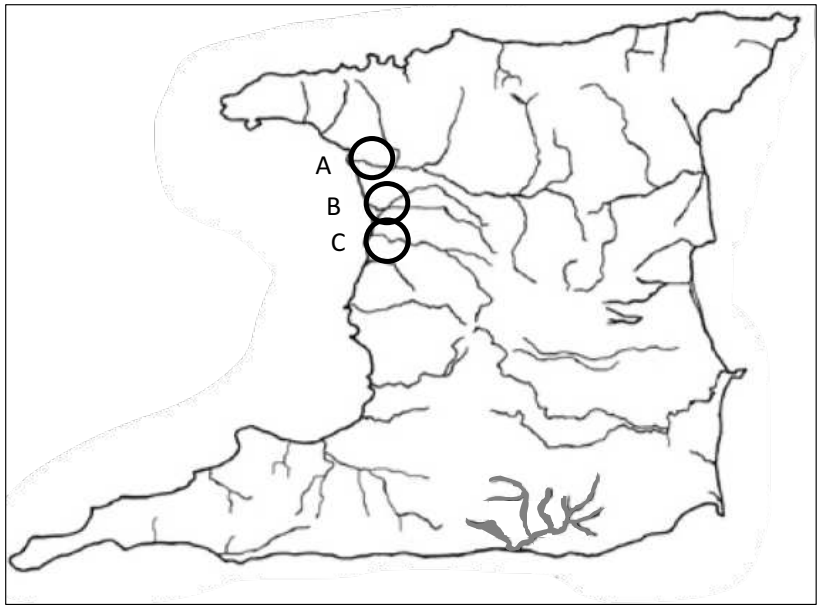

Figure 1.1- SAMPLING LOCATIONS: Map of sampling sites in Trinidad are indicated by circles: A=Caroni, B=Cunupia and C=Caparo

Host	River	Location (DD)	Sample Size
P. reticulata	Caparo	10.5174, -61.4319	20
P. reticulata, P. picta	Caroni	10.6191, -61.4269	20, 14
P. reticulata, P. picta	Cunupia	10.5622, -61.4179	20, 3
P. picta	Caroni	10,5621, -61.4659	12
P. picta	Cunupia	10.5547, -61.4380	11

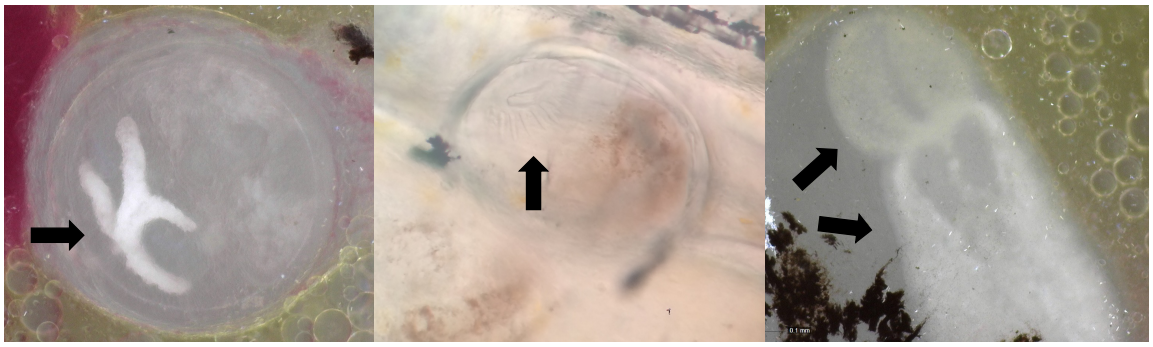

Figure 1.2- MORPHOSPECIES CLASSIFICATION: a) Morphospecies 1 has "X" shaped excretory ducts (arrow), and a spherical cyst shape. b) Morphospecies 2 has oral spines (arrow). c) Morphospecies 3 has distinct anterior and posterior body regions indicated by the black arrows

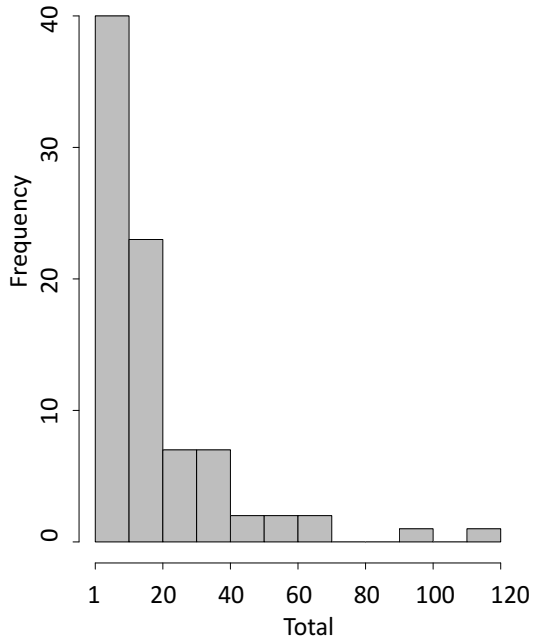

Figure 1.3- METACERCARIAL AUNDANCE ACROSS ALL INDIVIDUALS: Metacercarial abundance across all individuals followed a Poisson distribution, with most individuals harboring few parasites and a few harboring many

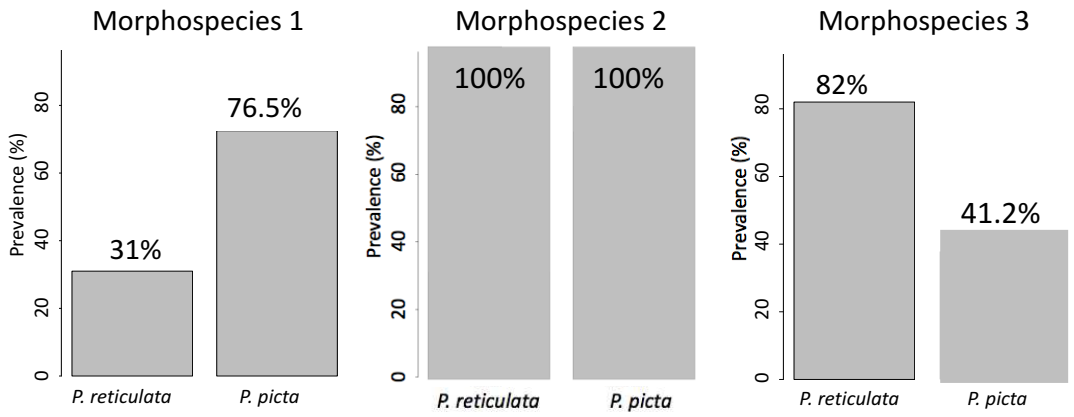

Figure 1.4- PREVALENCE OF MEATCERCARIAL MORPHOSPECIES IN *P. RETICULATA* AND FROM FRESHWATER SITES: Shown is the percentage of fish sampled with at least one parasite present.

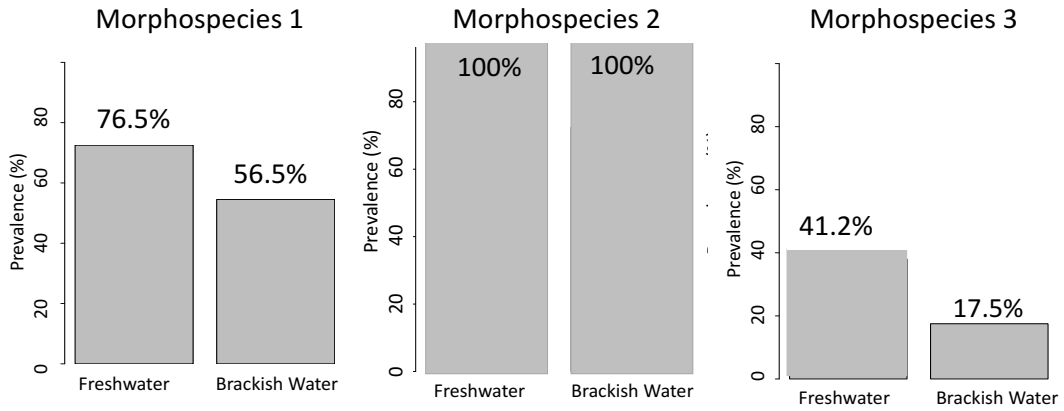

Figure 1.5- PREVALENCE OF METACERCARIAL MORPHOSPECIES IN *P. PICTA* FROM FRESH AND BRACKISH WATER: Shown is the percentage of fish sampled with at least one parasite present.

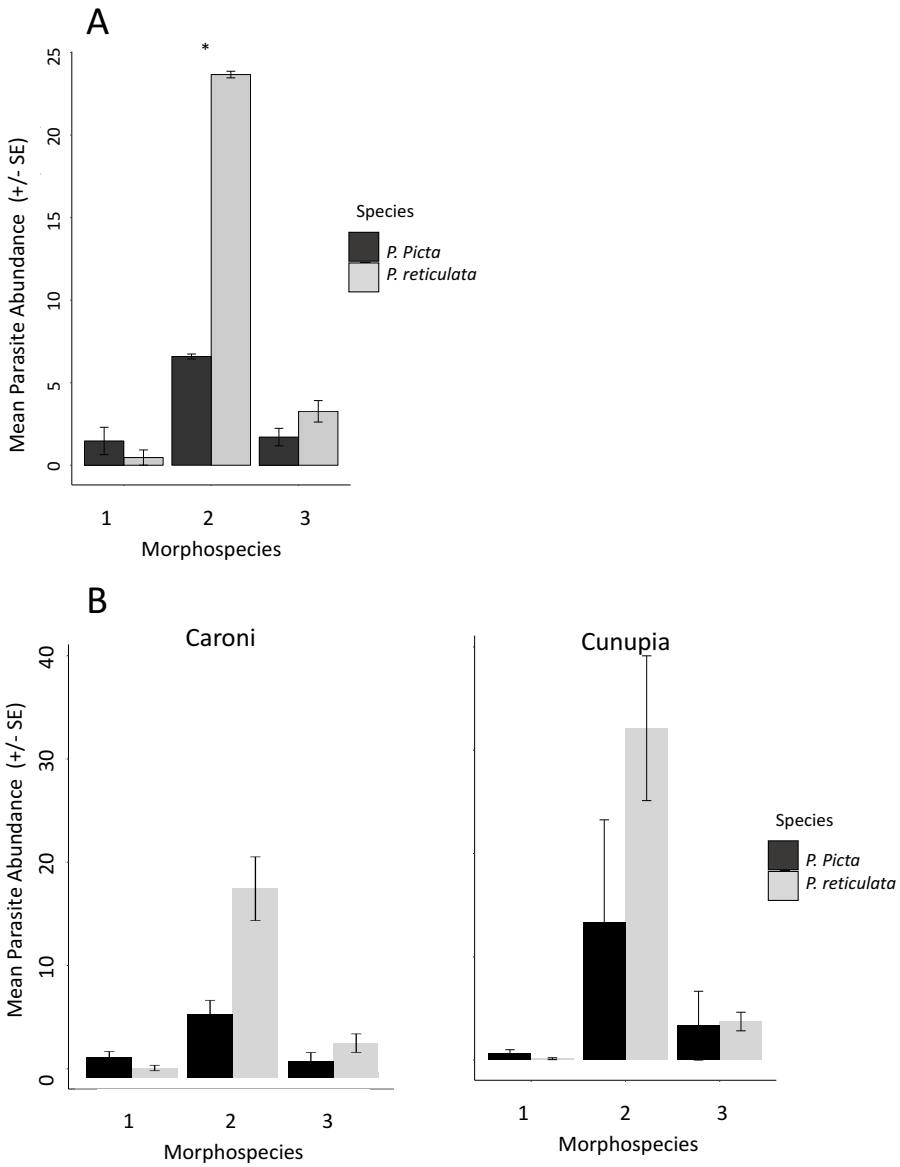

Figure 1.6- MEAN METACERCARIAL ABUNDANCE OF *P. PICTA* AND *P. RETICULATA* IN FRESHWATER BY MORPHOSPECIES: *=p-value< 0.0001. Graph A depicts overall metacercarial abundance and graph B depicts abundance by population.

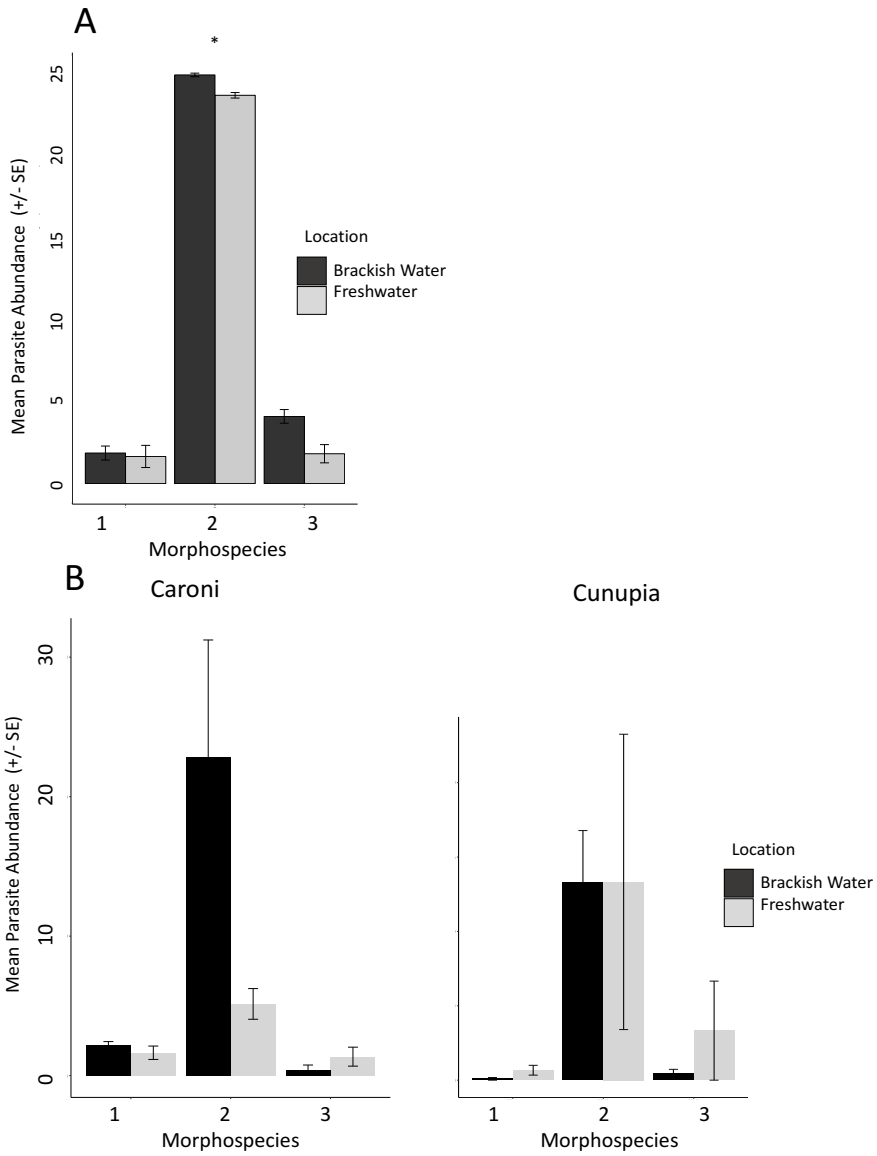

Figure 1.7- MEAN METACERCARIAL ABUNDANCE IN *P. PICTA* FROM FRESH AND BRACKISH WATER: *=p-value<0.0001. Graph A depicts overall metacercarial abundance and graph B depicts abundance by population.

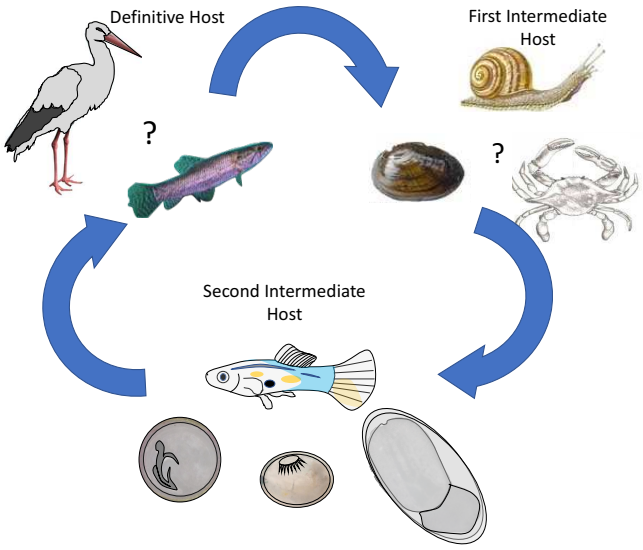

Figure 1.8- GENERALIZED DIGENEAN TREMATODE LIFE CYCLE WITH TWO INTERMEDIATE HOSTS: In the Trinidadian river systems *P. reticulata* and *P. picta* are second intermediate hosts for multiple digenean trematodes. However, definitive and first intermediate host species are unknown

CHAPTER 2: DO TREMATODE PARASITES SHAPE THE RANGE LIMITS OF TRINIDADIAN GUPPIES?

INTRODUCTION

All species exhibit geographic range limits, or geographical boundaries beyond which they are not found. Range limits are complex in origin and understanding their causes and consequences are of major importance in the field of evolutionary ecology (Barber et al., 2000; Sexton et al. 2009). While some species have abrupt changes in their distribution that are associated with specific environmental features, others appear to have a gradual waning of potential habitat that they occupy (Gaston, 2009). Because populations at the edges may experience unfavorable conditions, understanding how these edges are defined can give insight into the limits of population growth and sources of natural selection Identifying the abiotic and biotic factors that impose physiological limits on contemporary time scales, can also inform the selective pressures that prevent adaptation beyond the current boundaries of a population's geographic distribution (Bozick and Real, 2015).

Many biotic and abiotic factors are responsible for the observed patterns of species distribution (Case and Taper 2000; Holt 2003; Price and Kirkpatrick, 2008). While these biotic and abiotic factors are often examined in isolation, they can also interact on many levels to influence the habitats and communities occupied (Dunson and Travis 1991; Warner et al., 1991). For example, interactions between pH or salinity can alter the structure of aquatic communities in a variety of different habitats (Dunson and Travis 1991; Selleslagh and Amara, 2008; Jovem da Silva Azevedo et al.; 2015 McCreadie and Alder, 2012). Yet, most studies examining a role for biotic factors in shaping species distributions have focused on either competition or predation

(Dunson and Travis 1991; Holt and Barfield 2009), whereas the role of parasites has largely been ignored (Biers 2003).

Parasites can limit host ranges through a number of mechanisms that act either independently or through interactions with abiotic conditions (e.g. Holt 2003; Holt et al. 2005). Host species may actively avoid certain habitats to reduce parasite exposure or abundance. For example, several fish species actively avoid parasites through microhabitat selection (Poulin and FitzGerald, 1989), shifting position in the water column (Karvonen, 2004), or through shoaling behavior (Mikheev et al. 2013). Alternatively, if stressful abiotic conditions near the host's range boundary reduce immunity, higher rates and prevalence of parasitism could negatively impact host population growth and locomotor performance (Lafferty and Kuris 1999; Kiesecker and Skelly 2001; Deaton, 2009, Iwanowicz, 2011 and Stephenson et al., 2006). For example, Santos et. al (2011) found that increasing metacercarial abundance can negatively impact swimming behaviors. Similarly, Briers (2003) found that freshwater snails had higher parasite abundances at the range boundary where populations are calcium limited.

Salinity is an abiotic environmental factor that limits the distribution of both plants and animals (Nielsen et al., 2003; Josefson, 2016; Cornelia et al., 2011). Salinity is thought to be especially important in restricting the distribution of freshwater fish (Bruno et al., 2013). When freshwater fish are exposed to an increasing amount of salt in their environment many hormonal and physiological changes take place which can alter biotic interactions (Gonzalez, 2012; McCormick, 1996; Kumai and Perry, 2012). For example, a major hormone involved in osmoregulation is cortisol (McCormick, 1996). Cortisol has also been linked to behavioral changes in fishes and has been shown to decrease host immune response to parasites and other pathogens (Espelid et al., 1996) by influencing lymphocyte numbers and antibody production

capacity (Barton, 1991). For aquatic organisms that encounter a salinity gradient or fluctuating salinity levels these changes in immunity pose interesting questions about potential interactions with parasites and shaping of range limits.

STUDY SYSTEM

Poecilia reticulata on the island of Trinidad are an ideal model organism for ecological and evolutionary studies (Magurran, 2005), and for the study of parasite-host interactions (Dargent et. al, 2013). In Trinidad, the Northern Range mountains form the headwaters of numerous rivers that flow to the Atlantic and Caribbean Oceans. As these mountain streams reach coastal areas the salinity of the water gradually increases. *P. reticulata* is found in nearly every freshwater stream in Trinidad (Magurran, 2005; Torres-Dowdall et al., 2013), but *P. reticulata* populations are completely absent when any salt is present in the water (Torres-Dowdall et al., 2013). Such patterns are surprising, because *P. reticulata* is known to physiologically tolerate high levels of salinity (Shikano and Fujio, 1997) and has successfully colonized brackish waters in other geographic locations (Magurran, 2005). Such observations suggest other factors may be influencing the avoidance of brackish water environments.

Digenean trematodes are the major parasite of *P. reticulata* in Trinidadian rivers (Chapter 1) and could interact with salinity to restrict *P. reticulata* distributions to freshwater environments. Digenean trematodes are a group of flatworms that utilize multiple hosts to complete their life cycle, and the same morphospecies infect both *P. reticulata* and *Poecilia picta* at the freshwater-brackish water interface (Chapter 1).

In order to determine if *P. reticulata* avoids brackish water, because of higher parasite loads, we experimentally exposed *P. reticulata* from fresh water to field-collected brackish and freshwater assumed to contain live digenean metacercariae. If higher parasitic infections prevent

P. reticulata from dispersing into brackish water, then exposure to field-collected brackish water will lead to higher parasite abundance compared to field-collected freshwater.

METHODS AND MATERIALS

FISH COLLECTION

Butterfly nets were used to collect forty female *P. reticulata* from the banks of four different freshwater sites from three independent river systems on the island of Trinidad: Caparo (10.5174, -61.4319 DD), Caroni (10.6191, -61.4269 DD), the Cunupia (10.5547, -61.4380 DD), and El Cedro (Figure 1.1). The Caparo, Caroni, and Cunupia sample sites were near but upstream from the freshwater-brackish water boundary. The ElCedro site was approximately 20km upstream of the brackish water boundary of the Caroni river sampling site. Unlike the three other sites, previous sampling of *P. reticulata* from the El Cedro river revealed no digenean trematode metacercariae among the 30 fish sampled (Robison et al. in prep).

EXPERIMENTAL DESIGN

Fish were transported from the field to the laboratory, where they were acclimated for 24 hours in glass tanks containing five gallons of filtered lab-prepared freshwater. Fish from each river location were then randomly split into two groups, one designated as a freshwater control group (n=20) and the other a brackish water exposure group (n=20). After the initial 24 hour acclimation time, the brackish-water exposure groups were gradually acclimated to increasing amounts of salinity at 5ppt per day, for a total of six days, using lab-filtered salt water. The freshwater group also received equal volume changes of filtered and prepared fresh water. Once the brackish water exposure groups were acclimated to 30ppt salinity they were exposed to unfiltered water (5 gallons) collected from the Caroni river, which measured 30-32ppt salinity. Freshwater individuals were exposed to unfiltered freshwater collected from the Caroni. Fish

were exposed to this freshly collected river water each day for a total of seven days. Mortality in each group was monitored and recorded. To control for population density between the treatment tanks in response to mortality, if a fish died in one treatment group, an individual in the control group was chosen at random and removed.

All fish that survived the freshwater and brackish water exposure were euthanized on the seventh day using MS-222. Weight was obtained utilizing an Ohaus SP-602 Scout Pro Digital Balance, and standard length was obtained by placing the individual on a ruler and taking a digital picture with an imbedded scale. We used the software package ImageJ (Rueden et al. 2017) to calculate standard length (cm). Fin clippings of pectoral and caudal fins were obtained along with skin scrapings and whole gill squash preps to analyze external parasites. Internal parasite abundance was analyzed through squash preps of abdominal internal structures that were categorized by tissue type (Yanong, 2003; Leung and Poulin, 2011; Weber and Govett, 2009). All tissues were examined for parasite prevalence and mean abundance. Internal tissues examined utilizing microscopy were GI tract, liver and gonads. Metacercariae were categorized by morphospecies described in Chapter 1 (Figure 1.2).

STATISTICAL ANALYSIS

All analyses were performed using R 3.3.1 open source software. The data were non-normally distributed (Figure 2.1) and thus a generalized linear model was used with a Poisson distribution for each analysis. In order to test the fixed effect of brackish water exposure total parasite abundance with all morphospecies combined was used as a response variable with population and the interaction of treatment and population as predictor variables. To test the how each morphospecies varied between treatments, we then ran the same model for each morphospecies separately.

RESULTS

FISH MORPHOMETRICS

Female *P. reticulata* weight from the Caparo river had an average weight of 0.18g (±0.01), Caroni 0.15g (±0.009) and Cunupia 0.12g (±0.01). Standard length of *P. reticulata* from the Caparo river was 2.4cm (±0.05), Caroni 2.27cm (±0.04) and the Cunupia 2.2cm (±0.04).

MORTALITY

All populations exposed to brackish water suffered at least fifty percent mortality. Fish from El Cedro, Caparo, Caroni, and Cunupia suffered 80%, 70%, 60%, and 50% percent mortality rates, respectively (Figure 2.2). Mortality rates were highest between days 5 and 7 of river collected brackish water exposure. In contrast, there was almost no mortality in the freshwater exposure group. Only one individual died in the freshwater exposure group during the acclimation period. No individuals died in response to exposure to field collected freshwater.

METACERCARIAL ABUNDANCE

Due to their high mortality rate and reduced sample size, the El Cedro population was removed from further analyses of metacercarial abundance (Figure 2.2). When assessing the effect of treatment on the total parasite abundance we found a significantly higher metacercarial abundance in fish exposed to brackish water compared to the freshwater exposed individuals ($z=6.06$, $df=1$, $p<0.0001$, see Figure 2.3). Further, we found that this pattern was consistent for each river population. However, the degree of difference between the treatments across each population varied, resulting in a significant population by treatment interaction ($z=-7.2$, $df=2$, $p<0.0001$; Figure 2.4).

While morphospecies 2 ($z=1.9$, $df=1$, $p=0.05$) and 3 ($z=5.57$, $df=1$, $p<0.0001$) both showed a significant increase in the brackish water treatment, morphospecies1 showed no difference

between treatments (Figure 2.5). These differences between morphospecies varied amongst populations (Caroni: $z=1.11$, $df=2$, $p<0.01$; Cunpia: $z=-1.23$, $df=2$, $p<0.05$, see figure 2.6); however, there was no indication of a population interaction with treatment.

DISCUSSION

An important role for parasites in shaping host species range limits and distributions has largely been overlooked despite the fact that parasites can result in major physiological costs and reduce host population growth rates (Boulinier et al., 2001; Briers, 2003; Altizer et al., 2003). The impact of parasites on host populations may be particularly strong at range boundaries, where suboptimal abiotic conditions can weaken host immunity (Briers, 2003; Lazzaro and Little, 2009; Martin et al., 2010). Here, we show that digenean trematodes are a common parasite of *P. reticulata* and that they contribute to shaping *P. reticulata* range limits by restricting them to freshwater environments. Under laboratory conditions we found that *P. reticulata* readily acclimates to filtered brackish water with little to no mortality. However, exposure to unfiltered brackish water collected directly from the river results in an increase in total metacercarial abundance and high mortality. These results are consistent with the hypothesis that salinity is associated with higher parasite loads and may shape the range boundary of Trinidadian guppies. Similar patterns have been described for explaining the geographic range limits of freshwater snails in relation to their parasites (Biers, 2003). However, the causal links between salinity, parasites, and *P. reticulata* distributions remain unresolved.

Few empirical studies have been able to experimentally test the causes of range limits in mobile aquatic animals because they do not easily lend themselves to transplant experiments beyond their native ranges (Brown, 1984; Angert and Schemske, 2005; Gaston, 2009; Sexton et al., 2009). Here we mimicked range expansion by exposing lab acclimated guppies to field-

collected brackish water. Compared to the control group which was exposed to field-collected freshwater from the same river, *P. reticulata* exposed to field collected brackish water suffered higher mortality and higher parasite loads (Figures 2.2–2.4).Because the guppies were able to successfully acclimate to 30 ppt salinity during the acclimation period with no mortality (Figure 2.2), salinity alone was not responsible for the mortality and the parasites, or a combination of salinity stress and parasite load was responsible for the higher mortality rates.

The present study has a few weaknesses. We could not determine the difference in parasite load in fish that died following brackish water exposure because the dead fish rapidly decomposed. Similarly, while we have no reason to suspect a bias, we cannot be certain that guppies in the brackish water group did not already have higher parasite loads compared to the control group. Lastly, we assume the repeated exposure of field collected water exposed the guppies to the free living cercaria of the trematode parasites. Unfortunately, we were not able to verify the presence of cercariae in the river water despite several attempts. Though the increase in total metacercariae indicates that parasitism was a factor, it is also possible that pollutants and other aquatic pathogens in the river water may also have been introduced and contributed to the patterns observed. Further studies will be needed to understand the linkages between salinity, the abundance of the parasites, and the consequences of infection.

Theoretical models suggest that abrupt geographic range boundaries can occur in species that behaviorally avoid unfavorable environments (e.g. Holt et al., 2005). Past work has shown that throughout Trinidadian rivers, *P. reticulata* actively avoids even small increases in salinity caused by fluctuating tidal and rainfall effects, such that the sharp range boundary is likely due to behavioral avoidance (Torres-Dowdall et al., 2013). If the association between salinity and parasites provide a reliable cue, such results could explain how behavioral avoidance would

evolve. Indeed, other studies have found that fish will exhibit behavioral changes to avoid parasitism (Poulin and FitzGreald, 1989; Karvonen, 2004), suggesting they have the ability to recognize and respond to appropriate cues. However, it is less clear whether exposure to salinity reduces *P. reticulata* immunity (e.g. Espelid et al.; 1996; Barton, 1991), or whether there are simply more parasites in brackish water. Among the parasites, morphospecies 2 and 3 were responsible for the higher parasite abundances in the brackish water exposure group (Figure 2.5), thus it is possible these species are more common compared to freshwater sites. Previous work has shown that morphospecies 2 is also the most common parasite of *P. reticulata* in freshwater sites on Trinidad (Chapter 1). Morphologic speciation has its limitations and in particular it is unclear if morphospecies 2 in brackish water is genetically distinct from the freshwater form, which would have made the exposure to these parasites novel to *P. reticulata* and consequently more vulnerable to infection (Lyles and Dobson, 1993). Nevertheless, future work will need to explore the degree to which *P. reticulata* in fresh and saltwater can fight off infection of invading cercariae (Mostowy and Engelstadter, 2011), and whether guppies avoid the cercariae, the salinity, or their combination.

FIGURES

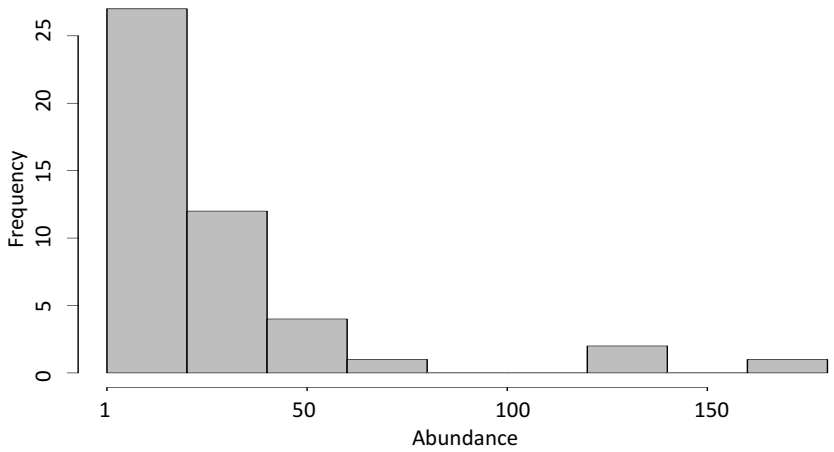

Figure 2.1– METACERCARIAL ABUNDANCE ACROSS ALL INDIVIDUALS: Metacercarial counts followed a Poisson distribution, with most individuals harboring few parasites and a few harboring many.

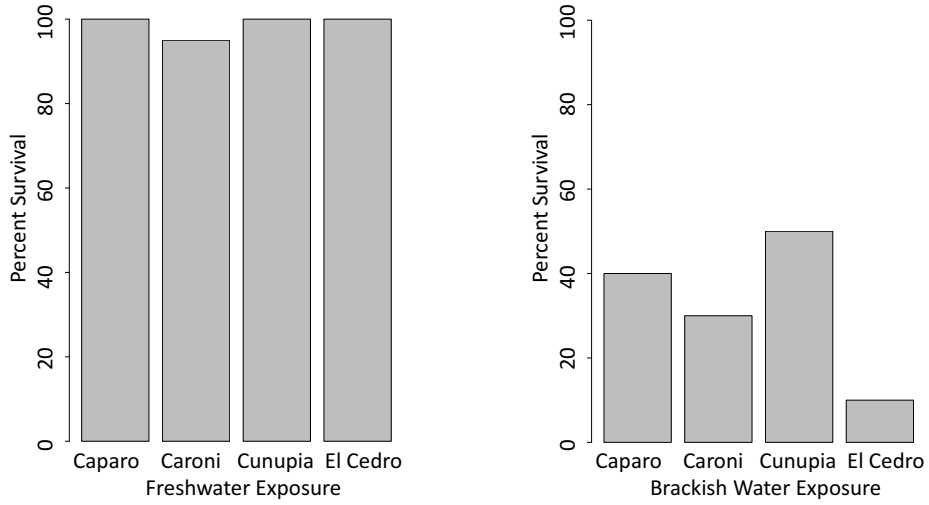

Figure 2.2 – SURVIVAL RATES AMONGST EXPOSURE GROUPS: All deaths occurred after exposure to stream collected brackish water. Mortality was highest in the El Cedro population, though all populations suffered at least a 50% mortality rate

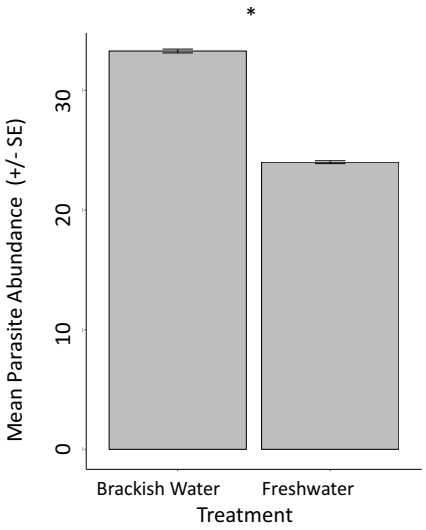

Figure 2.3- OVERALL METACERCARIAL ABUNDANCE IN SALT EXPOSED FISH: Graph depicting overall change in metacercarial abundance between the fresh and brackish water exposure. *=p-value <0.001.

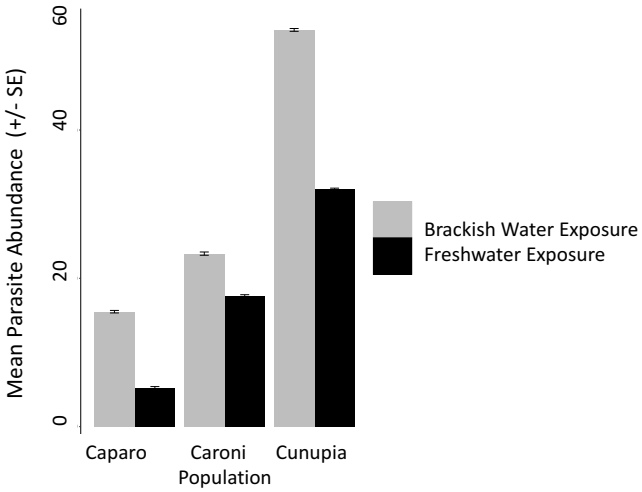

Figure 2.4- METACERCARIAL ABUNDANCE BY POPULATION ACROSS EXPOSURE GROUPS: All populations experienced significant increase in mean parasite abundance ($p<0.001$).

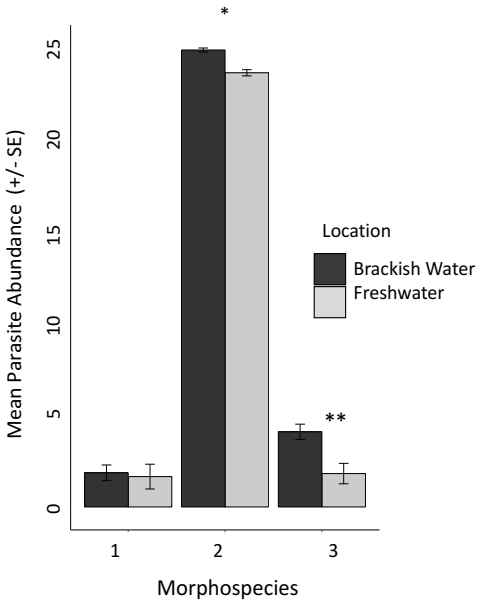

Figure 2.5– METACERCARIAL MORPHOSPECIES ABUNDANCE ACROSS EXPOSURE GROUPS: While all morphospecies show a trend of increasing with brackish water treatment, morphospecies 2 and 3 were significantly increased in brackish water exposed fish. *=$p<0.05$,**=$p<0.0001$.

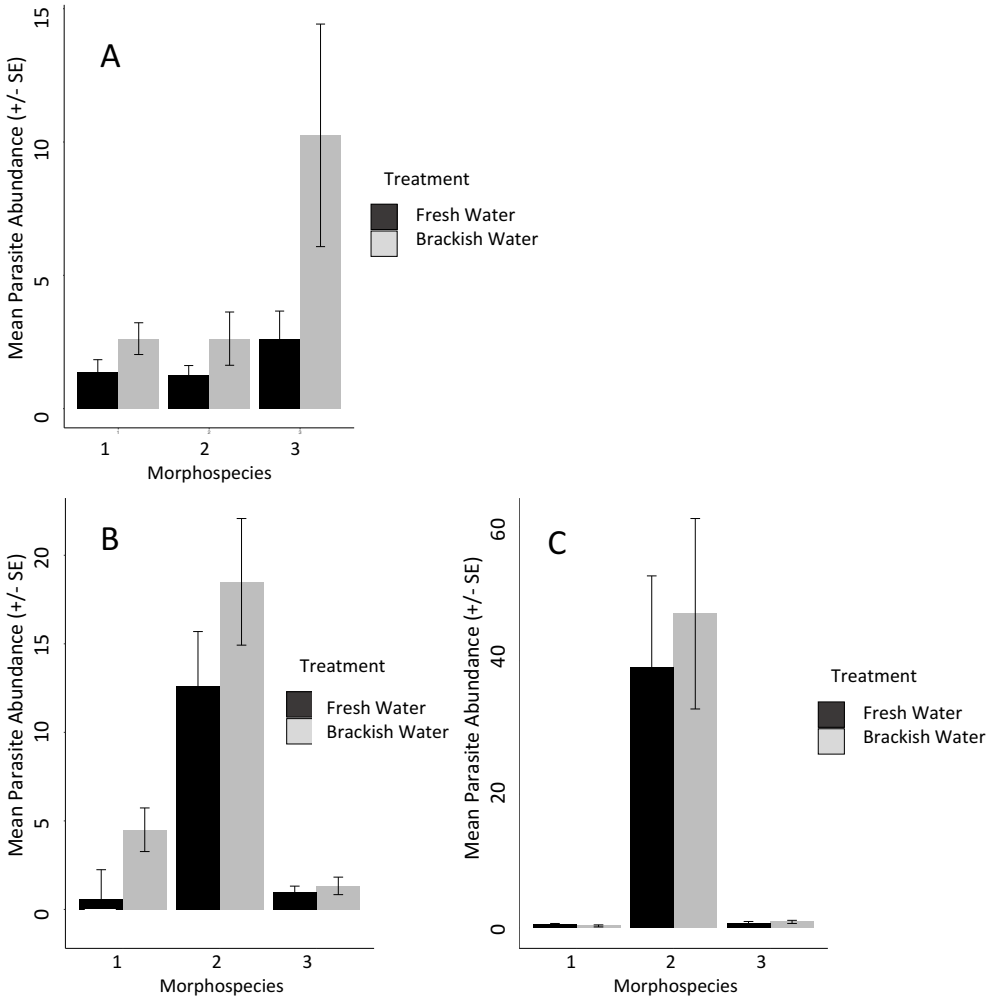

Figure 2.6– MEAN VISCERAL MORPHOSPECIES ABUNDANCE IN FRESH AND BRACKISH WATER: Panel A represents the Caparo, B – Caroni and C – Cunupia rivers.

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). 1999. Case Studies in Environmental Medicine (CSEM) Cadmium Toxicity. Atlanta: US Department of Health and Human Services; 1999 may.
2. Altizer, S., Harvell, D. and Friedle, E. (2003). Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution, 18(11), 589-596.
3. Angert, A. L. and Schemske, D. W. (2005). The Evolution of Species' Distributions: Reciprocal transplants across the elevation ranges of *Mimulus cardinalis* and *M. Lewisii*. Evolution, 59(8), 1671-1684.
4. Arendt, J.D. and Reznick, D.N. 2005. Evolution of juvenile growth rates in female guppies (Poecilia reticulata): predator regime or resource level? Proceedings of the Royal Society B: Biological Sciences. 272(1560):333-337.
5. Barber, I., Hoare, D. and Krause, J. 2000. Effects of parasites on fish behavior: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries. 10:131-165.
6. Barton, B. A. 1991. Physiological changes in fish from stress in aquaculture with
7. Bernot, R.J. 2003. Trematode infection alters the antipredator behavior of pulmonate snail. Journal of the North American Benthological Society. 22(2):241-248
8. Blasco-Costa and I., Poulin, R. 2013. Host traits explain the genetic structure of parasites: a meta-analysis. Parasitology. 140, 1316-1322.
9. Borgsteede, F. H. 1996. The effect of parasites on wildlife. Veterinary Quarterly. 18(3): S138-140

10. Boschetto, C., Gasparini, C. and Pilastro, A. 2011. Sperm number and velocity affect sperm competition success in the guppy (Poecilia reticulata). Behavioral Ecology and Sociobiology. 65(4):813-821.

11. Boulinier, T., McCoy, K.D., and Sorci, G. 2001. Dispersal and parasitism. Dispersal (ed. By J. Colbert, E. Danchin, A.A. Dhondt and J.D. Nichols), pp. 169-179. Oxford University Press, Oxford, U.K.

12. Bozick, B.A. and Real, L.A. 2015. Integrating parasites and pathogens into the study of geographic range limits. Quarterly Review of Biology. 90(4): 361-380.

13. Briers, R. A. 2003. Range limits and parasite prevalence in a freshwater snail. Proceedings of the Royal Society of London: Biological Sciences, 270(2), S178-S180

14. Brooks, R. and Endler, J.A. 2001. Female Guppies Agree to Differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. Evolution. 55(8):1644-1655.

15. Brown, J.H. 1984. On the Relationship between Abundance and Distribution of Species. The American Naturalist. 124(2):255-279.

16. Bruno, D. O., Barbini, S. A., Diaz de Astarloa, J. M. and Martos, P. 2013. Fish
    a. abundance and distribution patterns related to environmental factors in a choked temperate coastal lagoon (Argentina). Brazilian Journal of Oceanography. 61(1):43-53.

17. Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. 1997. Parasitology Meets Ecology on its Own Terms: Margolis et al. Revisited. Journal of Parasitology. 83(4): 575-583.

18. Cable, J., van Oosterhout. 2007. The role of innate and acquired resistance in two natural

populations of guppies (*Poecilia reticulata*) infected with the ectoparasite Gyrodactylus turnbulli. Biological Journal of the Linnean Society. 90, 647-655.

19. Combes, C. 2005. The Art of Being a Parasite. Chicago (IL): The University of Chicago Press Books. 280 p.

20. Cornelia, J., Moller, L. F. and Kiorboe, T. 2011. Salinity Gradient of the Baltic Sea Limits the Reproduction and Population Expansion of the Newly Invaded Comb Jelly *Mnemiopsis leidyi*. PLoS ONE. 6(8): e24065

21. Cwiklinski, K., Dalton, J.P., Dufresne, P.J., La Course, J., Williams, D.J.L., Hodgkinson, J. and Paterson, S. 2014. The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biology. 16:71

22. Dargent, F., Torres-Dowdall, J., Scott, M. E., Ramnarine, I. and Fussmann, G. F. 2013. Can Mixed-Species Groups Reduce Individual Parasite Abundance? A Field Test with Two Closely Related Poeciliid Fishes (*Poecilia reticulata* and *Poecilia picta*). PLOS ONE. 8(2): e56789

23. Deaton, R. 2009. Effects of a parasitic nematode on male mate choice in a livebearing fish with a coercive mating system (western mosquitofish, *Gambusia affinis)*. Behavioural Processes. 80(1):1-6

24. Dosen, L.D. and Montgomerie, R. 2004. Mate preferences by male guppies (Poecilia reticulata) in relation to the risk of sperm competition. Behavioral Ecology and Sociobiology. 55(3):266-271.

25. Duran-Reynals, F. Tissue permeability and the spreading factors in infection. Bacteriological Reviews. 1942. 6(4): 197-252.

26. Dynesius, M. and Jansson, R. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. PNAS. 97(16):9115-9120.

27. Ebert, D. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 9, Population Dynamics and Community Ecology.

28. Endler, J.A. 1980. Natural selection on color patterns in Poecilia reticulata. Evolution. 34:76-91.

29. Espelid, S., Lokken, G.B., Steiro, K. and Bogwald, J. 1996. Effects of cortisol and stress on the immune system in Atlantic Salmon (Salmo salar L.). Fish and Shellfish Immunology. 6:95-110.

30. Evans, B. B. and Lester, J. G. 2001. Parasites of ornamental fish imported into Australia. Bulletin- European Association of Fish Pathologists. 21(2): 51-55.

31. Evans, J.P. and Magurran, A.E. 1999. Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. Animal Behaviour. 58(5):1001-1006.

32. Fried, B. and Graczyk, T.K., Advances in Trematode Biology. CRC Press, 1997. Print.

33. Galaktionov, K. V. and A. A. Dobrovol'skiĭ. *The Biology and Evolution of Trematodes: An Essay on the Biology, Morphology, Life Cycles, Transmission, and Evolution of Digenetic Trematodes*. Dordrecht: Kluwer Academic, 2003. Print.

34. Gaston, K. J. 2009. Geographic range limits of species. Proceedings of the Royal Society: Biological Sciences, 276, 1391-1393.

35. Gonzalez, R.J. 2012. The physiology of hyper-salinity tolerance in teleost fish: a review. Journal of Comparative Physiology. 182(3): 321-329.

36. Gotanda. K. M., Delaire, L. C., Raeymaekers, J. A. M., Perez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussmann, G. F. and Hendry, A. P. 2013. Adding parasites to the guppy-predation story: insights from field surveys. Oecologia. 172, 155-166.

37. Harris, P. D. and Lyles, A. M. 1992. Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on Guppies (*Poecilia reticulata*) in Trinidad. Journal of Parasitology. 78(5): 912-914.

38. Harvell, C.D., Mitchell, C.E., Ward, J.D., Altizer, S., Dobson, A.P., Ostfeld, R.S. and Samuel, M.D. 2002. Climate warming and disease risks for terrestrial and marine biota. Science. 296(5576):2158-2162.

39. Heads, M. 2015. The relationship between biogeography and ecology: envelopes, models, predictions: Biogeography and ecology. Biological Journal of the Linnean Society. 115(2):456-468.

40. Hellard, E., Fouchet, D., Vavre, F. and Pontier, D. 2015. Parasite-Parasite Interactions in the Wild: How to Detect Them? Trends in Parasitology. 31(12):640-652.

41. Hoffman, G. L. Parasites of North American freshwater fishes. Ithaca, NY: Cornell University Press, 1999. Print.

42. Holt, R. D., Keitt, T. H., Lewis, M. A., Maurer, B. A. and Taper, M. L. (2005). Theoretical models of species' borders: single species approaches. *Oikos*, *108*(1), 18-27.

43. Horwitz, P. and Wilcox, B.A. 2005. Parasites, ecosystems and sustainability: an ecological and complex systems perspective. International Journal for Parasitology. 35:725-732.

44. Hurd, H. 1990. Physiological and behavioural interactions between parasites and invertebrate hosts, Advances in Parasitology. 29:271-318.

45. Iwanowicz, D.D. 2011. Overview on the effects of parasites on fish health. Bridging America and Russia with Shared Perspectives on Aquatic Animal Health (ed. By R.C. Cipriano, A.W. Bruckner and I.S. Shchelkunov), pp. 176-184. Proceedings of the Third Bilateral Conference between Russia and the United States, 12-20 July, 2009, held in Shepherdstown, West Virginia. Khaled bin Sultan Living Oceans Foundation, Landover, MD, USA.

46. Jimenez-Garcia, M. I. and Vidal-Martinez, V.M. Temporal Variation in the Infection Dynamics and Maturation Cycle of Oligogonotylus Manteri (Digenea) in the Cichlid Fish, '*Cichlasoma' Urophthalmus*, from Yucatan, Mexico. Journal of Parasitology. 91(5):1008-1014.

47. Jokela, J., Lively, C. M., Dydahl, M. F. and Fox, J. A. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biological Journal of the Linnean Society. 79 (1): 165-181.

48. Jokela, J., Taskinen, J., Mutikainen, P. and Kopp, K. 2005. Virulence of parasites in hosts under environmental stress: experiments with anoxia and starvation. OIKOS 108: 156-164.

49. Josefson, A. B. 2016. Species Sorting of Benthic Invertebrates in a Salinity Gradient – Importance of Dispersal Limitation. PLoS ONE. 11(12): e0168908.

50. Jovem da Silva Azevdo, D., Etham de Lucena Barbosa, J, Porto, D.E., Gomes, W.I.A.and Molozzi, J. 2015. Biotic or abiotic factors: which has greater influence in determining the structure of rotifers in semi-arid reservoirs?. Acta Limnologica Brasiliensia. 27(1): 60-77

51. Khan, R.A. 2012. Host-Parasite Interactions in Some Fish Species. Journal of Parasitology Research. 2012: 237280.

52. Klemme, I. and Karvonen, A. 2016. Learned parasite avoidance is driven by host personality and resistance to infection in a fish-trematode interaction. Proceedings of the Royal Society of London B: Biological Sciences. 283(1838):20161148.

53. Koprivnikar, J., Baker, R.L. and Forbes, M.R. 2007. Environmental factors influencing community composition of gastropods and their trematode parasites in southern Ontario. Journal of Parasitology. 93(5): 992-998.

54. Kumai, Y. and Perry, S. F. 2012. Mechanisms and regulation of Na+ uptake by freshwater fish. Respiratory Physiology and Neurobiology, 184(3):249-256.

55. Lafferty, K.D, and Morris, A.K. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology. 77(5): 1390-1397.

56. Lafferty, K.D. and Shaw, J.C. 2013. Comparing mechanisms of host manipulation across host and parasite taxa. Journal of Experimental Biology. 216:56-66.

57. Lazzaro, B. P. and Little, T. J. (2009). Immunity in a variable world. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364(1513), 15-26.

58. Lei, F. and Poulin, R. 2011. Effects of salinity on multiplication and transmission of an intertidal trematode parasite. Marine Biology. 158:995-1003.

59. Leung, T.L.F. and Poulin, R. 2011. Intra-host competition between co-infecting digeneans within a bivalve second intermediate host: Dominance by priority-effect or taking advantage of others?, International Journal for Parasitology. 41(3-4): 449-454.

60. Locke, S. A., McLaughlin, J.D. and Marcogliese, D.J. 2010. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea

(Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Molecular Ecology. 19: 2813-2827.

61. Lyles, A.M. and Dobson, A.P. 1993. Infectious Disease and Intensive Management: Population Dynamics, Threatened Hosts, and Their Parasites. Journal of Zoo and Wildlife Medicine. 24(3):315-326.

62. Magurran, Anne E. (2005). Evolutionary Ecology: The Trinidadian Guppy. New York: Oxford University Press.

63. Martin, L. B., Hopkins, W. A., Mydlarz, L. D. and Rohr, J. R. 2010. The effects of anthropogenic global changes on immune functions and disease resistance. Annals of the New York Academy of Sciences, 1195(1), 129-148.

64. Martinez, J. and Merino, S. 2011. Host-parasite interactions under extreme climatic conditions. Current Zoology. 57(3):390-405.

65. Maze-Guilmo, E., Blanchet, S., McCoy, K.D. and Loot, G. 2016. Host dispersal as the driver of parasite genetic structure: a paradigm lost? Ecology Letters. 19(3):336-347.

66. McCormick, S. D. 1996. Effects of Growth Hormone and Insulin-like Growth Factor I on Salinity Tolerance and Gill Na+, K+-ATPase in Atlantic Salmon (Salmo salar): Interaction with Cortisol. General and Comparative Endocrinology. 101: 3-11

67. McCormick, S.D., Moyes, C.D. and Ballantyne, J.S. 1989. Influence of salinity on the energetics of gill and kidney of atlantic salmon (*Salmo salar*). Fish Physiology and Biochemistry. 6(4): 243-254.

68. McCreadie, J.W. and Adler, P.H. 2012. The roles of abiotic factors, dispersal and species interactions in structuring stream assemblages of black flies (Diptera: Simuliidae). Aquatic Biosystems. 8:14.

69. Millar, N.P. and Hendry, A.P. 2012. Population divergence of private and non-private signals in wild guppies. Environmental Biology of Fishes. 94(3):513-525.

70. Minchella, D.J. and Scott, M.E. 1991. Parasitism: A cryptic determinant of animal community structure. Trends in Ecology and Evolution. 6(8):250-254.

71. Minguez, L., Buronfosse, T., Beisel, J-N. and Giamberini, L. 2012. Parasitism can be a confounding factor in assessing the response of zebra mussels to water contamination. Environmental Pollution. 162:234-240.

72. Moller, H. 1978. The effects of salinity and temperature on the development and survival of fish parasites. Journal of Fish Biology. 12(4):311-323.

73. Moore, J. 2002. Parasites and the Behavior of Animals. Oxford University Press.

74. Mostowy, R. and Engelstadter, J. 2011. The impact of environmental change on host – parasite coevolutionary dynamics. Proceedings of the Royal Society of London B: Biological Sciences. 278(1716): 2283-2359.

75. Mysterud, A., Qviller, L., Meisingset, E.L. and Viljugrein, H. 2016. Parasite abundance and seasonal migration in red deer. Oecologia. 180(2):401-407.

76. Nielsen, D. L., Brock, M. A., Rees, G. N. and Baldwin, D. S. 2003. Effects of increasing salinity on freshwater ecosystems in Australia. Australian Journal of Botany. 51:655-665.

77. Niewiadomska, K. and Pojmanska, T. 2011. Multiple strategies of digenean trematodes to complete their life cycles. Wiadomosci Parazytologiczne. 57(4): 233-241.

78. Paradis, E., Robert Baillie, S., Sutherland, W.J. and Gregory, R.D. 2002. Dispersal and spatial scale affect synchrony in metapopulation dynamics. Ecology Letters. 2(2):114-120.

79. Pedersen, A.B. and Antonovics, J. 2013. Anthelmintic treatment alters the parasite community in a wild mouse host. Biology Letters. 9, 20130205.

80. Picullel, B., Hoeksema, J.D. and Thompson J.N. 2008. Interactions of biotic and abiotic environmental factors in an ectomycorrhizal symbiosis, and the potential for selection mosaics. BMC Biology. 6:23.

81. Pietrock, M. and Marcogliese, D.J. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends in Parasitology. 19(7):293-299.

82. Poulin, R. 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. Ecology Monographs, 63:283-302.

83. Poulin, R., FitzGerald, G. J. 1989. Risk of parasitism and microhabitat selection in juvenile sticklebacks. Canadian Journal of Zoology. 67:14-18.

84. Price, T. D., Kirkpatrick, M. 2009. Evolutionarily stable range limits set by interspecific competition. Proceedings of the Royal Society B: Biological Sciences. 276: 1429-1434.

85. Probst, S. and Kube, J. 1999. Histopathological effects of larval trematode infections in mudsnails and their impact on host growth: what causes gigantism in *Hydrobia ventrose* (Gastropoda: Prosobranchia)?. Journal of Experimental Marine Biology and Ecology. 238(1):49-68.

86. Santos, E., Cunha, R. A., Santos, C. P. 2011. Behavioral responses of Poecilia vivipara (Osteichthyies: Cyprinodontiformes) to experimental infections of Acanthocollaritrema umbilicatum (Digenea: Cryptogonimidae). Experimental Parasitology. 127 (2):522-526.

87. Scott, M.E. and Anderson, R.M. 1984. The population dynamics of Gyrodactylus bullartarudis (Monogenea) within laboratory population of the fish host Poecilia reticulata. Parasitology. 89(Pt 1):159-194

88. Seghers, B.H. 1974. Geographic variation in the responses of guppies (Poecilia reticulata) to aerial predators. Oecologia. 14(1-2):93-98.

89. Selleslagh, J. and Amara, R. 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). Estuarine, Coastal and Shelf Science. 79:507–517.

90. Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics, 40.

91. Shikano, T., Fujio, Y. 1997. Successful Propagation in Seawater of the Guppy *Poecilia reticulata* with Reference to High Salinity tolerance at Birth. Fisheries Science. 63(4), 573-575.

92. Skrjabin, K. 1964. Trematodes of Animals and Man Volume XVII. Jerusalem:Israel Program for Scientific Translations.

93. Stephenson, J., Kinsella, C., Cable, J. and van Oosterhout, C. 2016. A further cost for the sicker sex? Evidence for male-biased parasite-induced vulnerability to predation. Ecology and Evolution. 6(8), 2506-2515.

94. Stephenson, J. F., van Oosterhout, C., Mohammed, R. S. and Cable, J. 2015. Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators. Ecology. 96(2), 489-498.

95. Stokke, B.G., Ratikainen, I.I., Moksnes, A., Roskaft, E., Schulze-Hagen, K., Leech, D, Moller, A.P. and Fossoy, F. 2018. Characteristics determining host suitability for a generalist parasite. Scientific Reports. 8:6285

96. Tempelton, C.N. and Shriner, W.M. 2004. Multiple selection pressures influence Trinidadian guppy (Poecilia reticulata) antipredator behavior. Behavioral Ecology. 15(4):673-678.

97. Thieltges, D. and Rick, J. 2006. Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode Renicola roscovita (Digenea: Renicolidae). Diseases of aquatic organisms. 73:63-68.

98. Thomas, F., Poulin, R., Meeus, T., Fuegan, J-F. and Renaud, F. 1999. Parasites and Ecosystem Engineering: What roles Could They Play? Oikos. 84(1):167-171.

99. Timi, J.T. and Poulin, R. 2003. Parasite community structure within and across host populations of a marine pelagic fish: how repeatable is it? International Journal for Parasitology. 33(12):1353-1362.

100. Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. and Kuris, A.M. 2003. Introduced species and their missing parasites. Nature. 421:628-630

101. Torres-Dowdall, J., Dargent, F., Handelsman, C. A., Ramnarine, I. W., Ghalambor, C. K. 2013. Ecological correlates of the distribution limits of two poeciliid species along a salinity gradient. Biological Journal of the Linnean Society. 108, 790-805.

102. Van der Putten, W.H., Macel, M. and Visser, M.E. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philosophical Transactions of the Royal Society B. 365(1549):2025-2034.

103. Warner, S. C., William, W. and Travis, J. 1991. Interaction of pH, density, and priority effects on the survivorship and growth of two species of hylid tadpoles. Oecologia 88:331-339.

104. Wassom, D.L., Krco, C.J. and David, C.S. 1986. I-E expression and susceptibility to parasite infection. Immunology Today. 8(2):39-43.

105. Weber, E.S. and Govett. 2009. Parasitology and Necropsy of Fish. Compendium. 31(2):E1-E7.

106. Webster, P. Monrad, J. Kapel, C.M.O., Kristensen A.T., Jensen, A.L. and Thamsborg, S.M. 2017. The effect of host age and inoculation dose on infection dynamics of *Angiostrongylus vasorum* in red foxes (*Vulpes vulpes*). Parasites and Vectors. 10:4

107. Windsor, D. A. 1998. Controversies in parasitology, most of the species on earth are parasites. International Journal for Parasitology. 28(12):1939-1941.

108. Wisz, M.S., Pottier, J., Kissling, W.D., Pellissier, L., Lenoir, J., Damgaard, C.F., Dormann, C.F., Forchhammer, M.C., Grytnes, J-A., Guisan, A., Heikkinen, R.K., Hoye, T.T., Kuhn, I., Luoto, M., Maiorano, L., Nilsson, M-C., Normand, S., Ockinger, E., Schmidt, N.M., Termansen, M., Timmermann, A., Wardle and D.A., Aastrup, P., Svenning, J-C. 2013. The role of biotic interactions in shaping distributions and realized assemblages of species: implications for species distribution modelling. Biological Reviews. 88(1):15-30.

109. Xavier, R., Faria, P. J., Paladini, G., van Oosterhout, C., Johnson, M. and Cable, J. 2015. Evidence for Cryptic Speciation in Directly Transmitted Gyrodactylid Parasites of Trinidadian Guppies. PLoS ONE. 10(1): e0117096.

110. Yanong, R.P.E. 2003. Necropsy techniques for fish. Seminars in Avian and Exotic Pet Medicine. 12(2): 89-105.